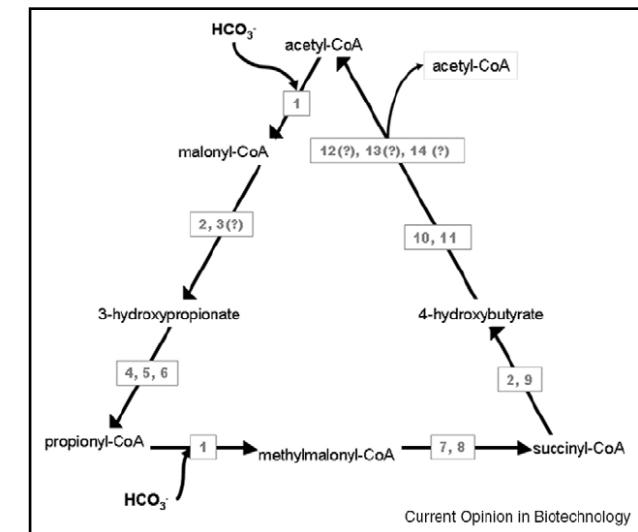
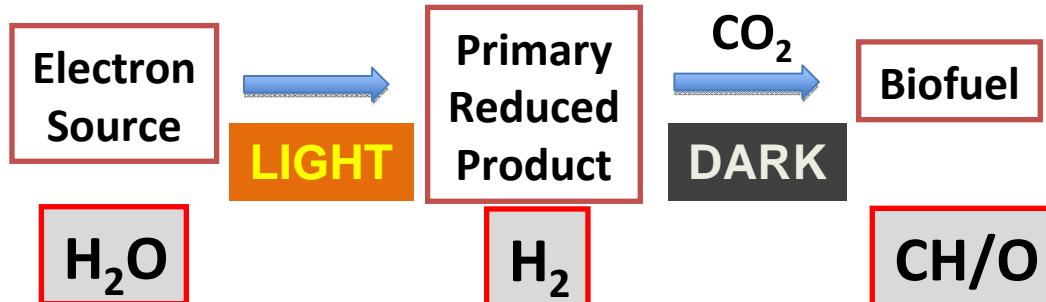


H_2 -driven CO_2 Fixation by Extremely Thermoacidophilic Archaea

Michael W.W. Adams, University of Georgia

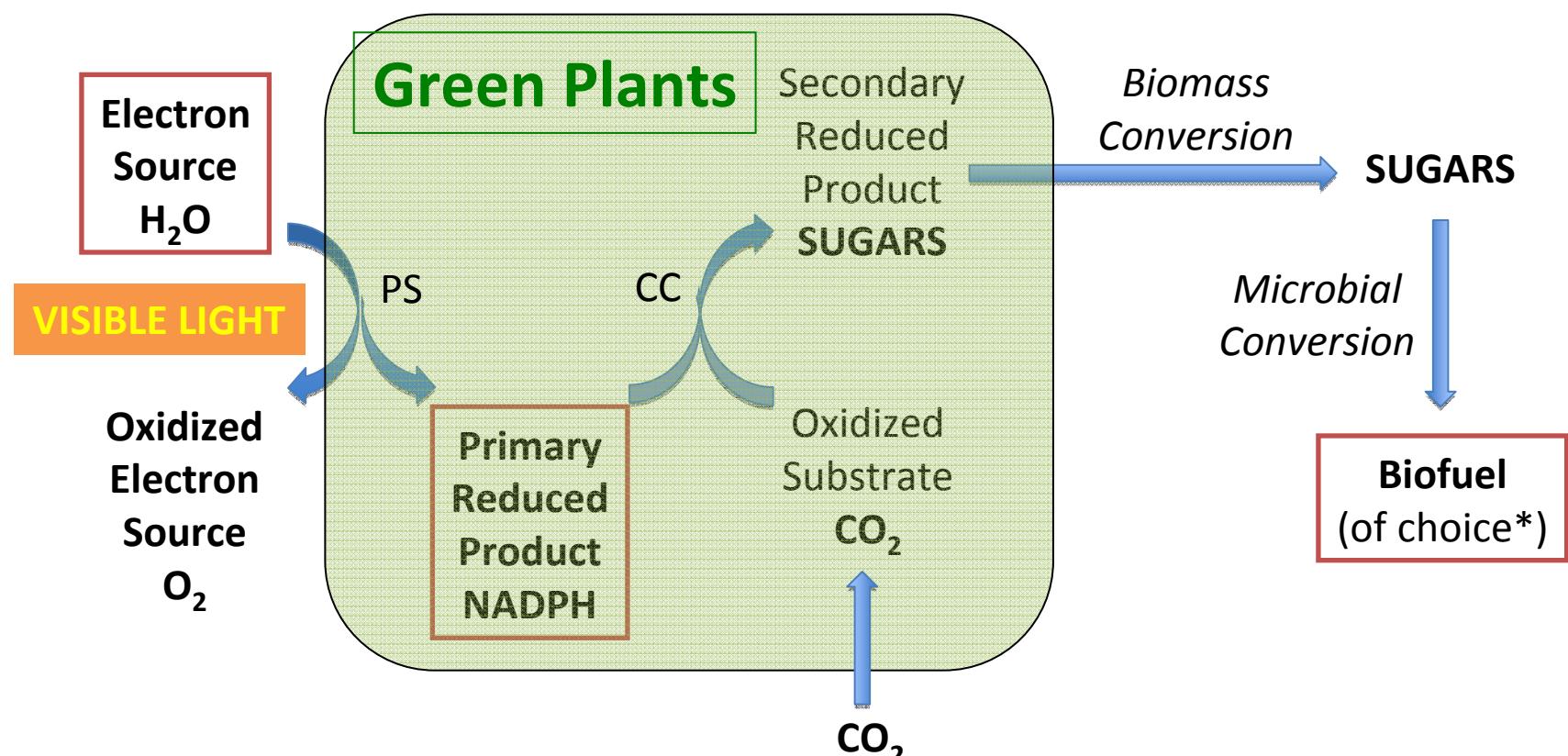
Robert M. Kelly, North Carolina State
University



SOLAR BIOFUEL PRODUCTION



SOLAR BIOFUEL PRODUCTION

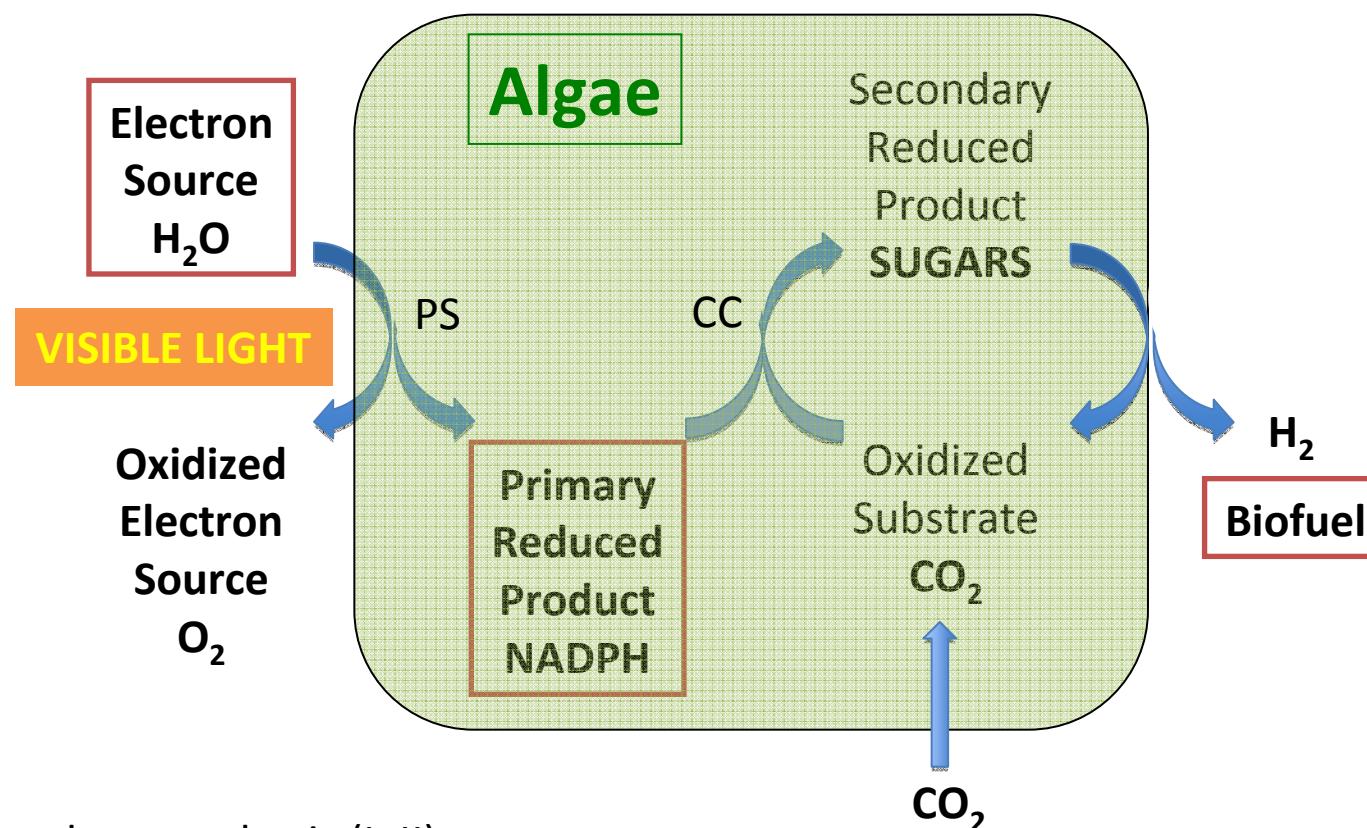


PS = photosynthesis (I+II)

CF = CO_2 fixation (Calvin cycle)

$*C_xH_y / C_aH_bO_c$

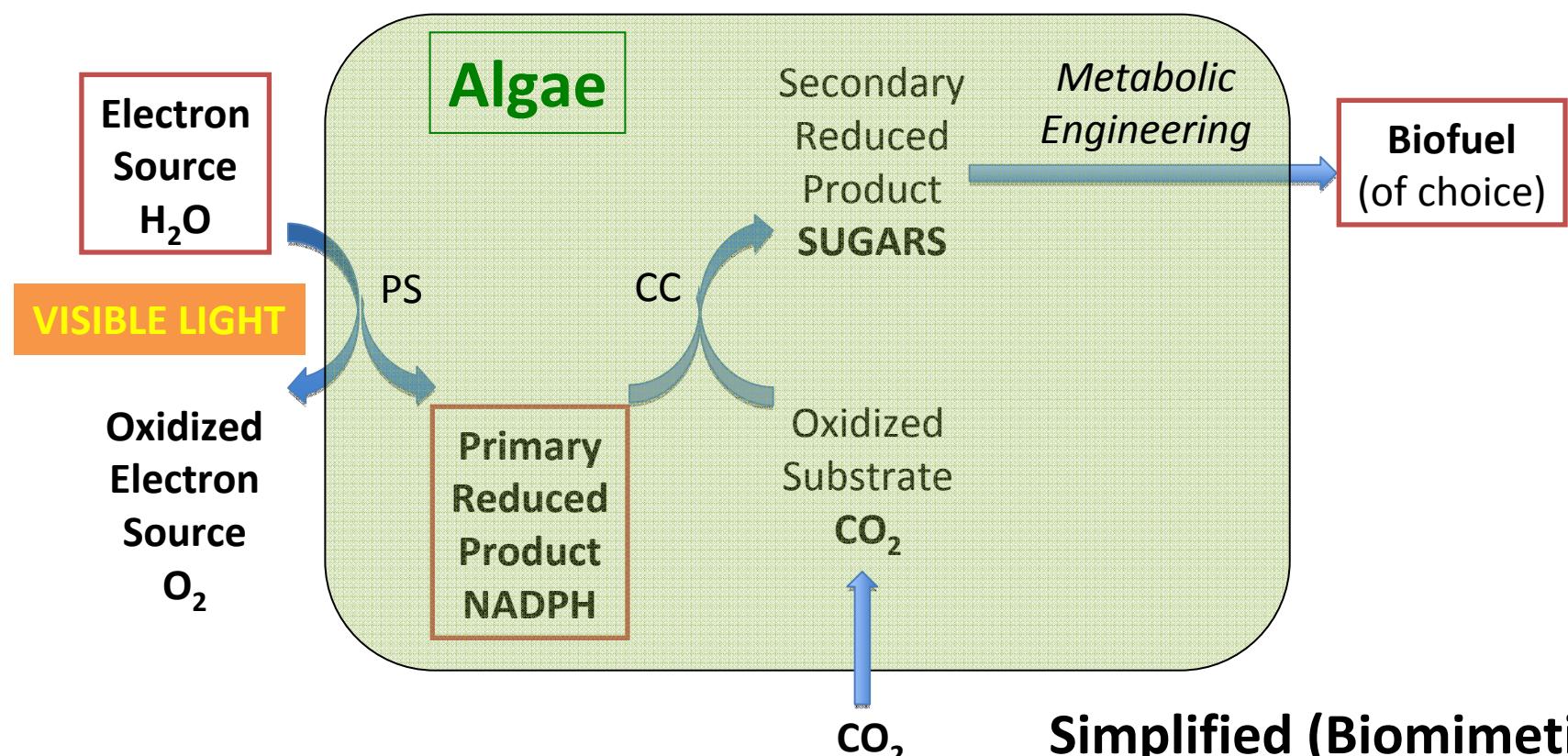
SOLAR BIOFUEL PRODUCTION



PS = photosynthesis (I+II)

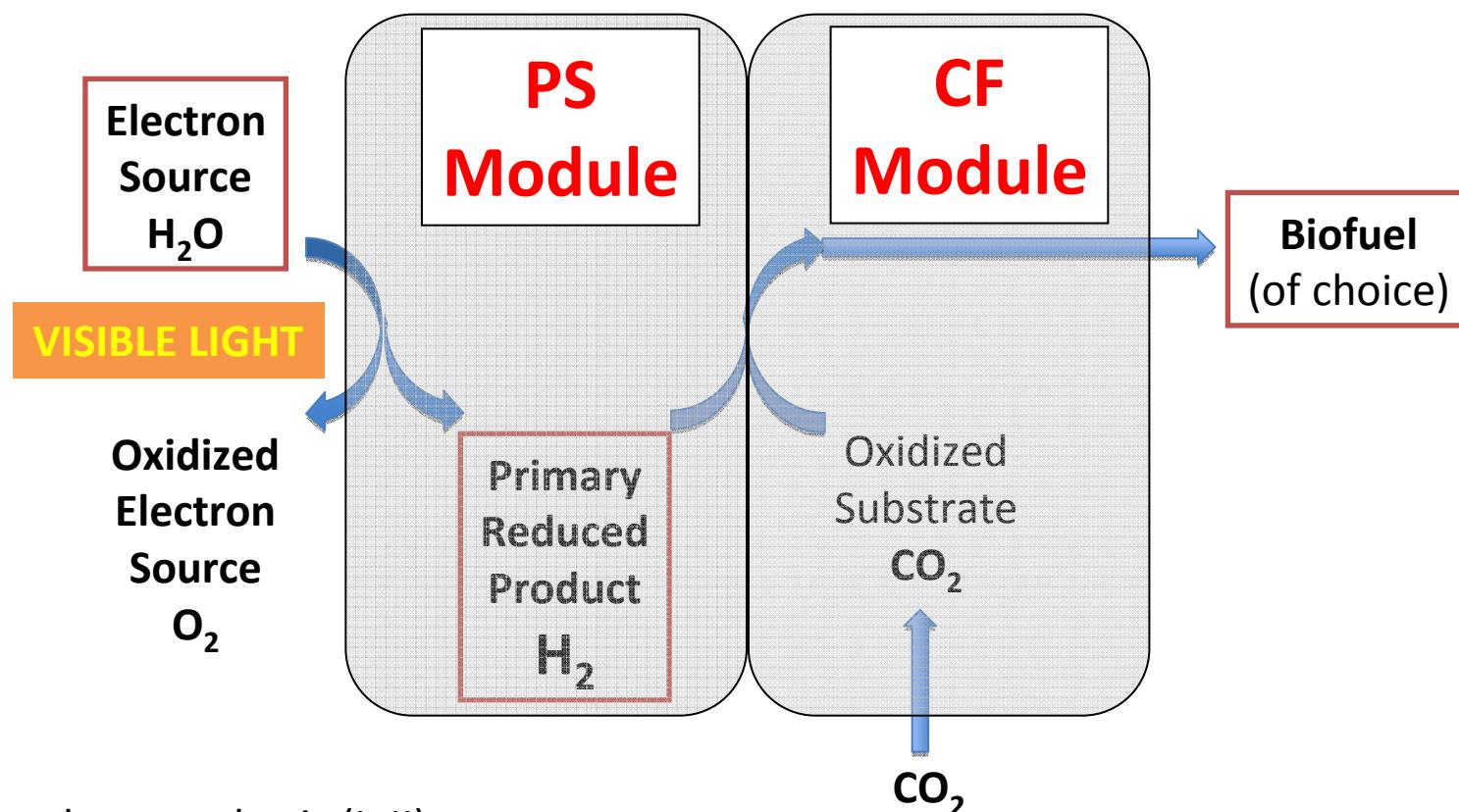
CF = CO₂ fixation (Calvin cycle)

SOLAR BIOFUEL PRODUCTION



Simplified (Biomimetic) Scheme?

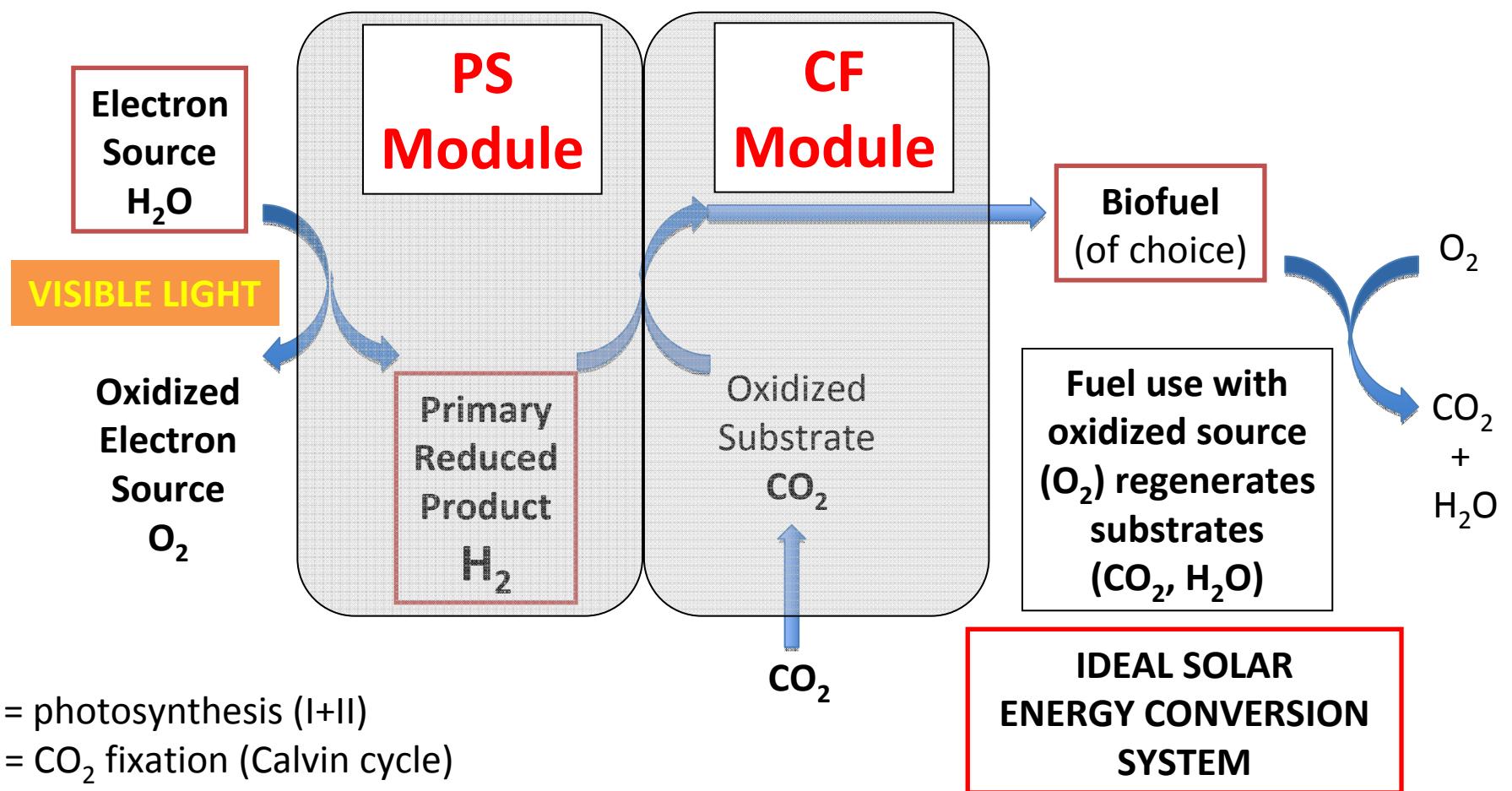
SOLAR BIOFUEL PRODUCTION



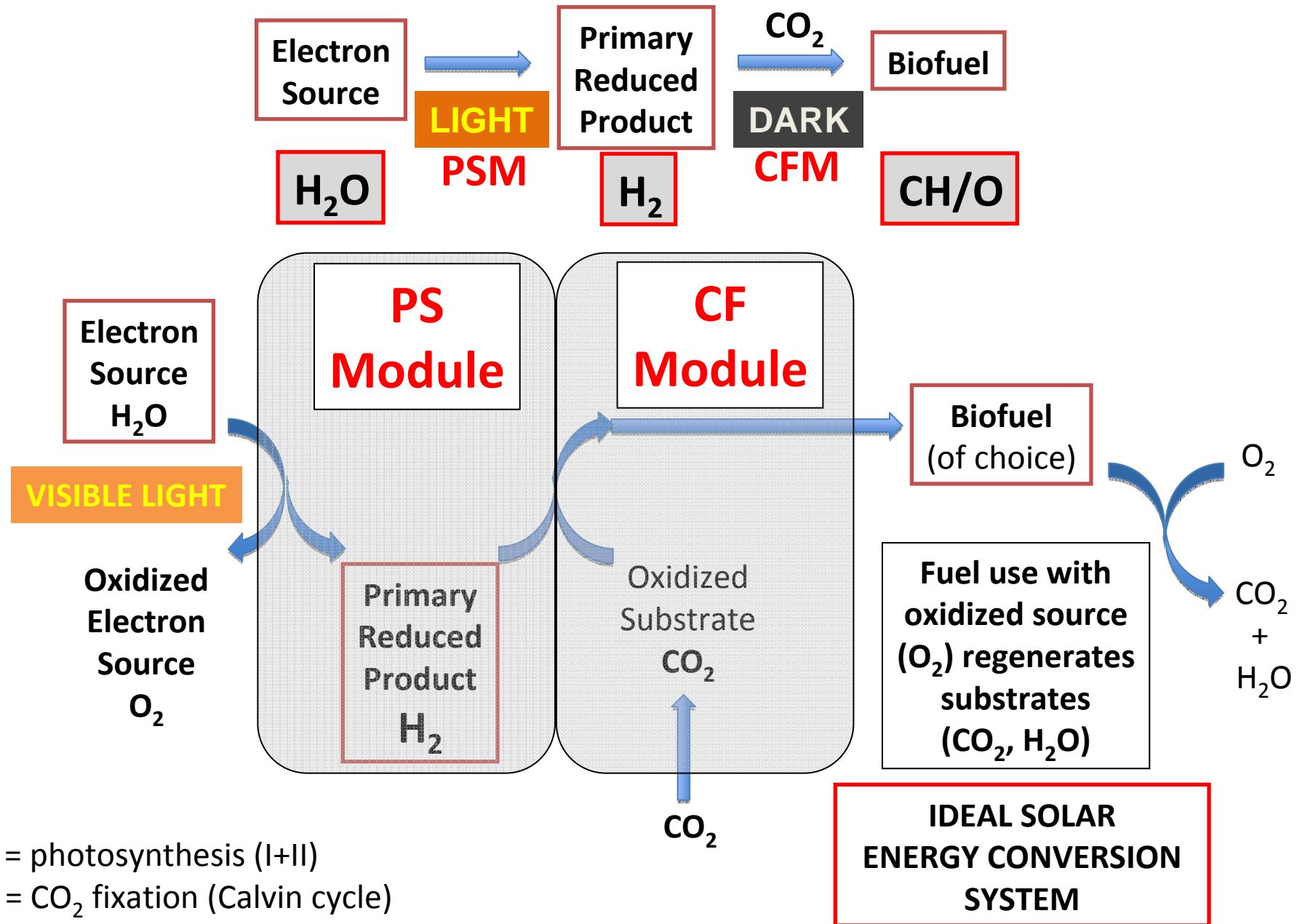
PS = photosynthesis (I+II)

CF = CO₂ fixation (Calvin cycle)

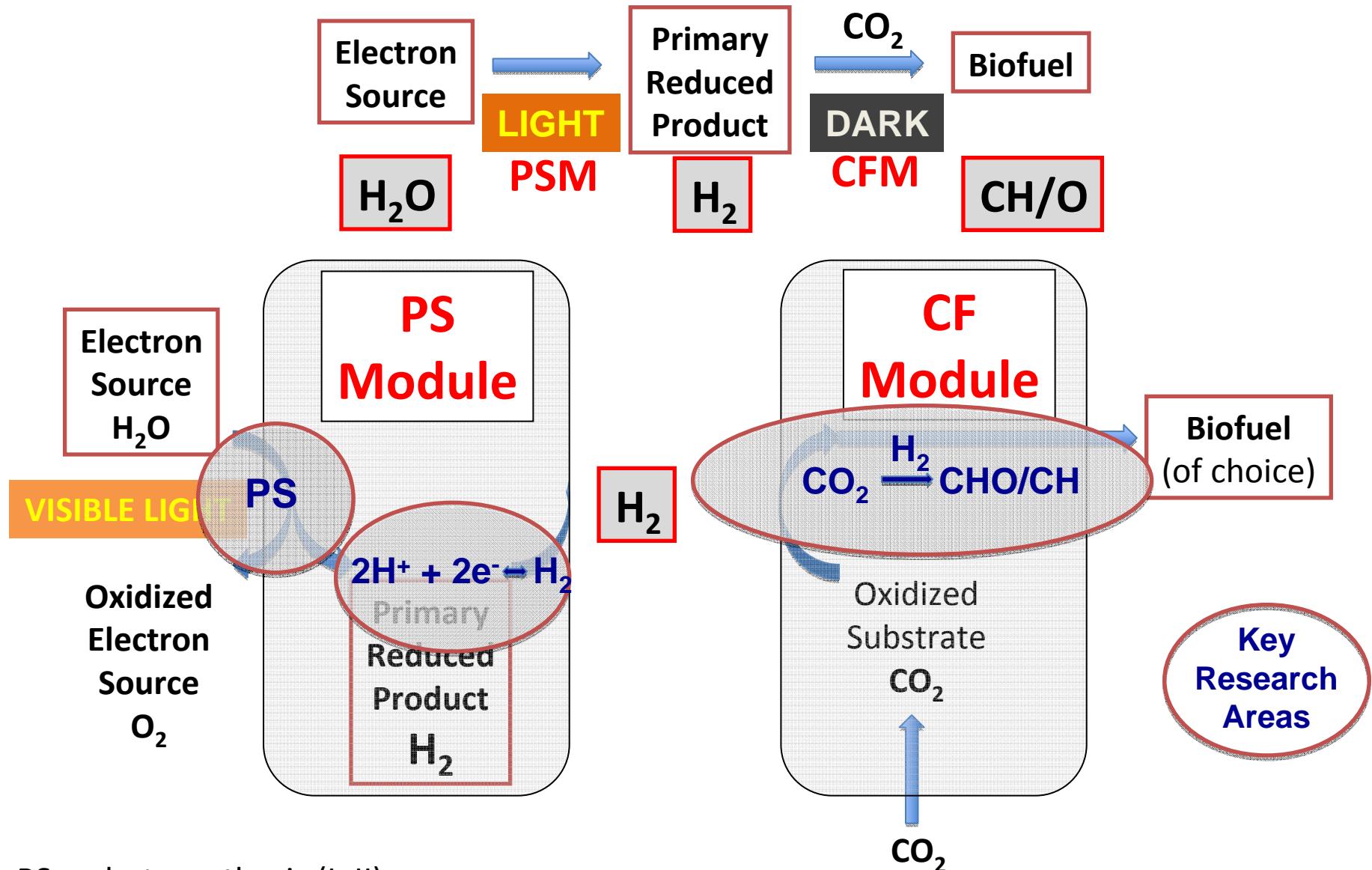
SOLAR BIOFUEL PRODUCTION



SOLAR BIOFUEL PRODUCTION



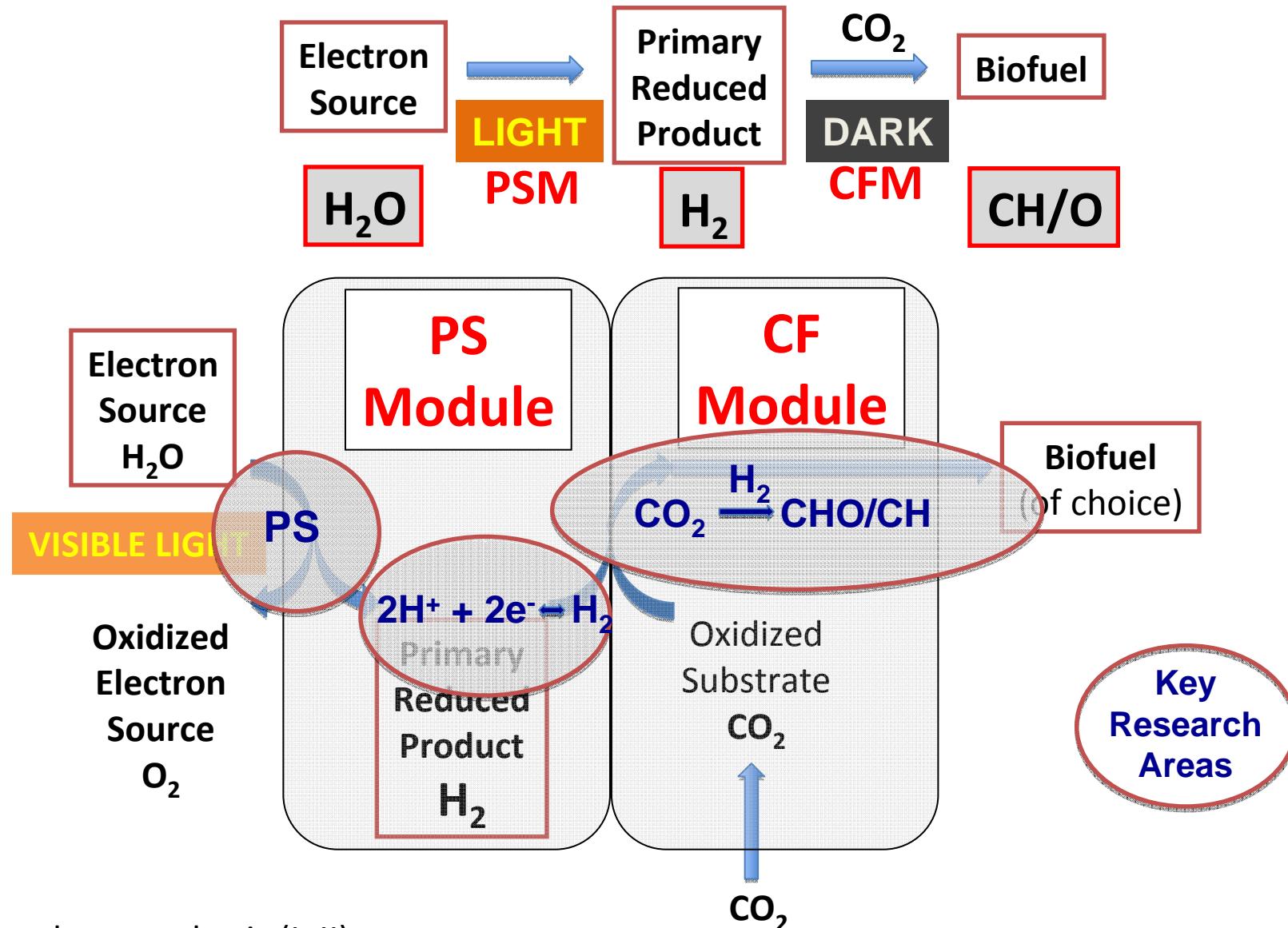
SOLAR BIOFUEL PRODUCTION



PS = photosynthesis (I+II)

CF = CO_2 fixation

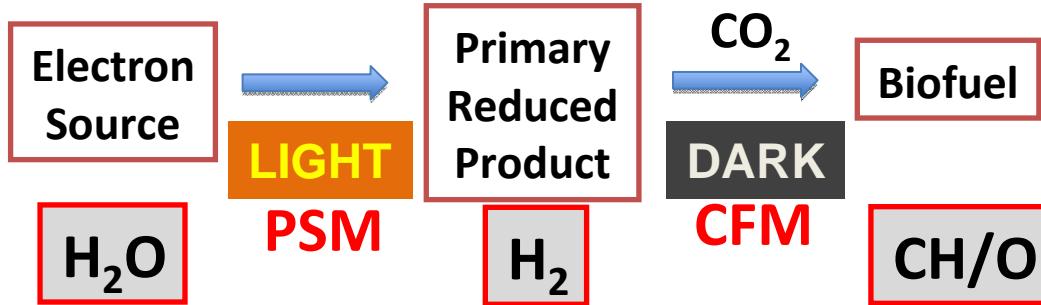
SOLAR BIOFUEL PRODUCTION



PS = photosynthesis (I+II)

CF = CO₂ fixation

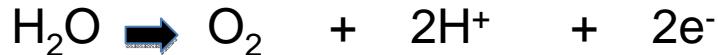
SOLAR BIOFUEL PRODUCTION



Key Biological Reactions

PSM:

1. Water photolysis (visible light):

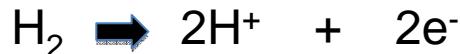


2. Hydrogen production

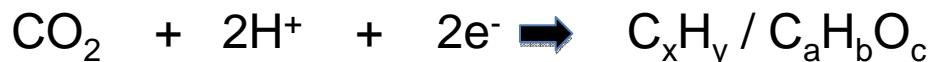


CFM:

1. Hydrogen activation



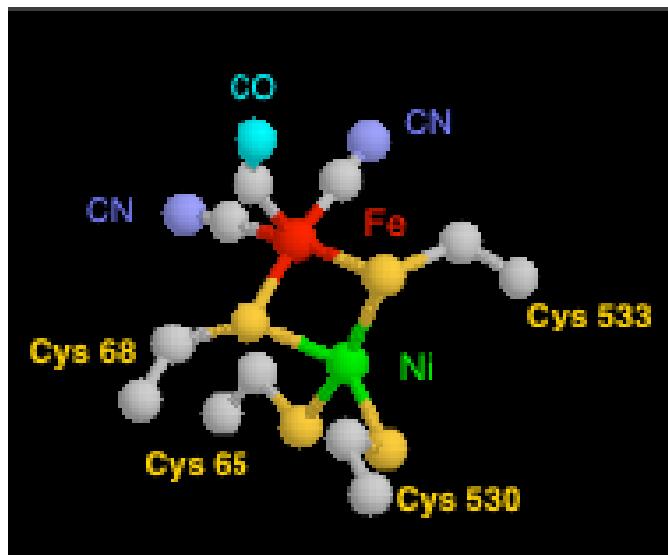
2. CO₂ to biofuel conversion (reduced carbon/CH/CHO)



Much higher energy yield using H₂ as reductant for CO₂ fixation to biofuel compared to sugar to biofuel

Structure/Function Studies of NiFe-hydrogenase

- Hydrogenase is not a 'commodity enzyme'
- Very complex maturation process *in vivo* to assemble complex, O₂-sensitive, NiFe-catalytic site (CO, CN ligands, etc.)
- No genetically-tractable organism that produces hydrogenase available for detailed structure/function studies
- **Recent breakthrough** – production of recombinant form of the NADPH-dependent *Pyrococcus* hydrogenase in *E. coli*

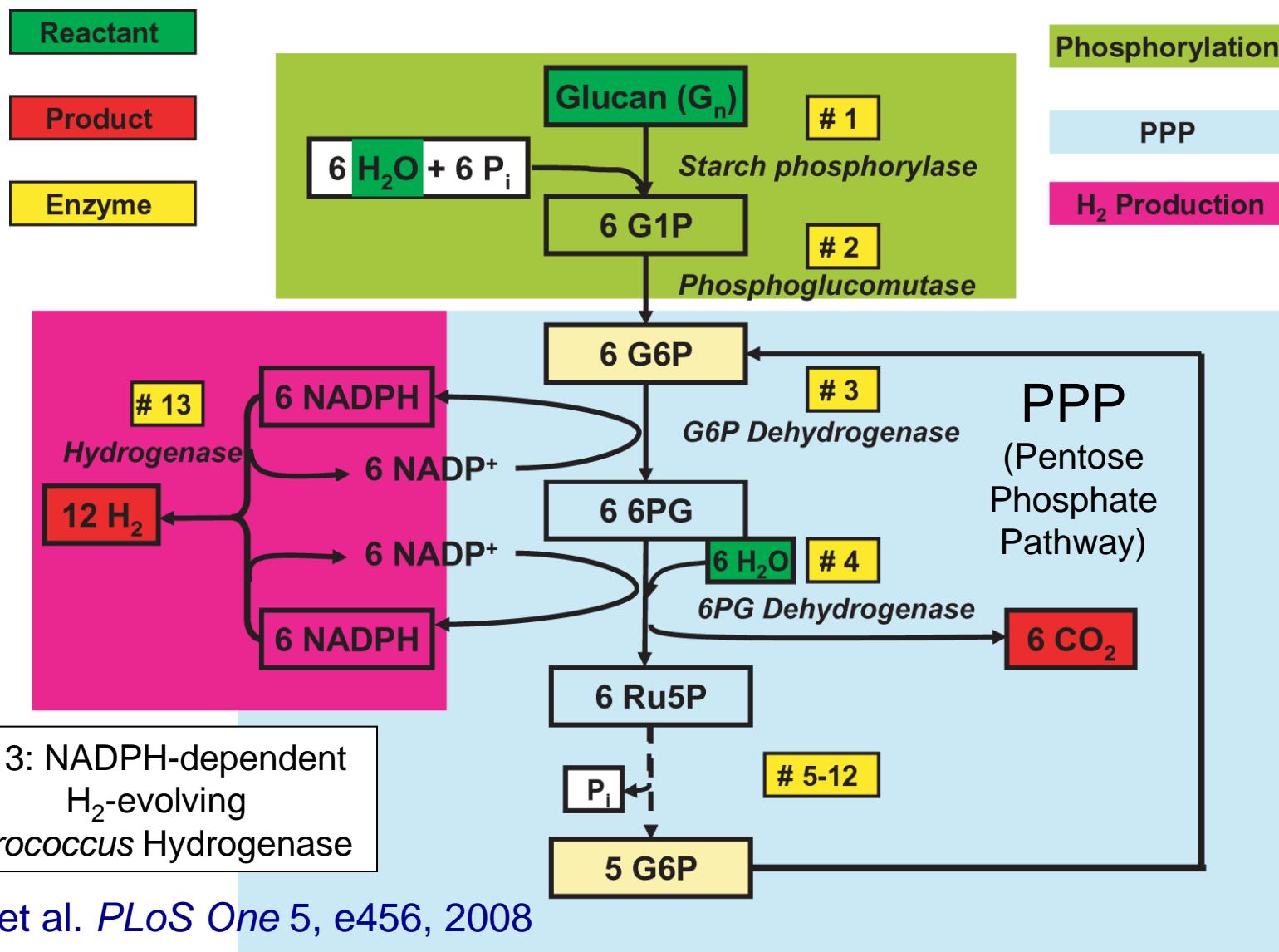


Fundamental structure/function studies to design:

- 'Minimal' hydrogenases (NiFe-site plus peptides)
- H₂ production and H₂ activation catalysts
- Decreased O₂-sensitivity
- Electron carrier specificity
- Pathways of electron transfer

Hydrogen Production from Polyglucose

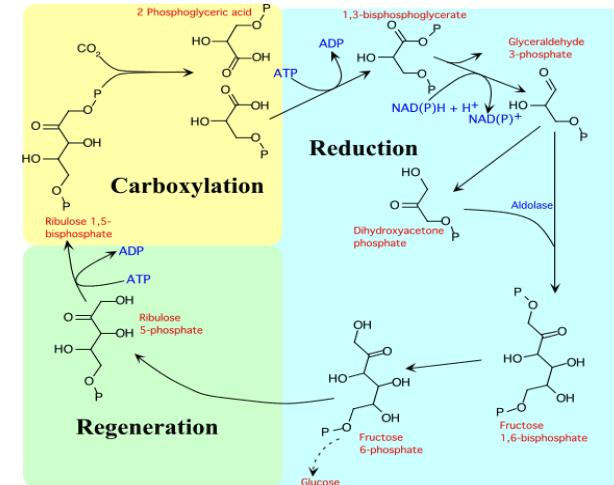
(13 enzymes, Pi, NADP – catalytic)



Microbial CO₂ Fixation Pathways

Calvin Cycle

plants, cyanobacteria, purple and green bacteria

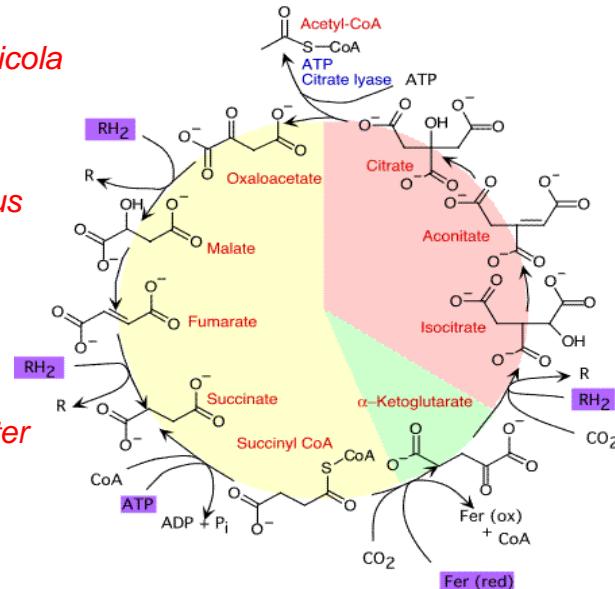


Reverse Tricarboxylic Acid Cycle

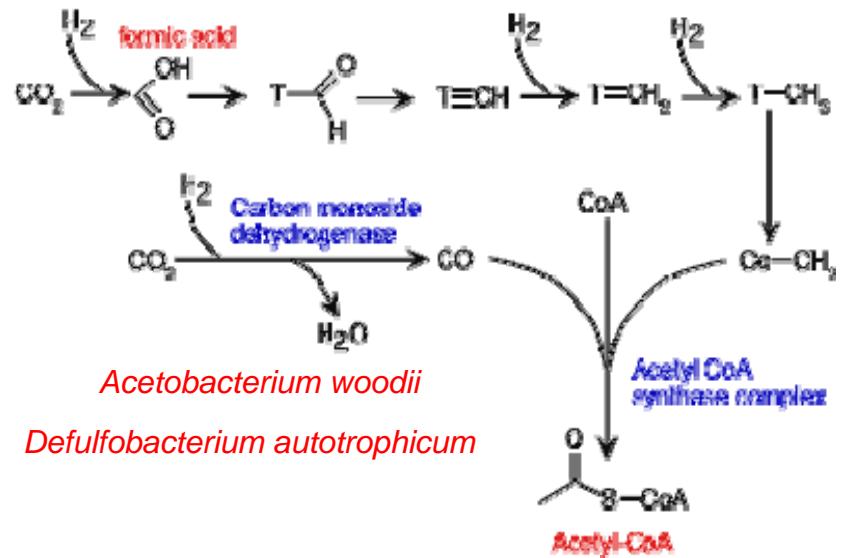
Chlorobium limicola

Desulfobacter hydrogenophilus

Hydrogenobacter thermophilus



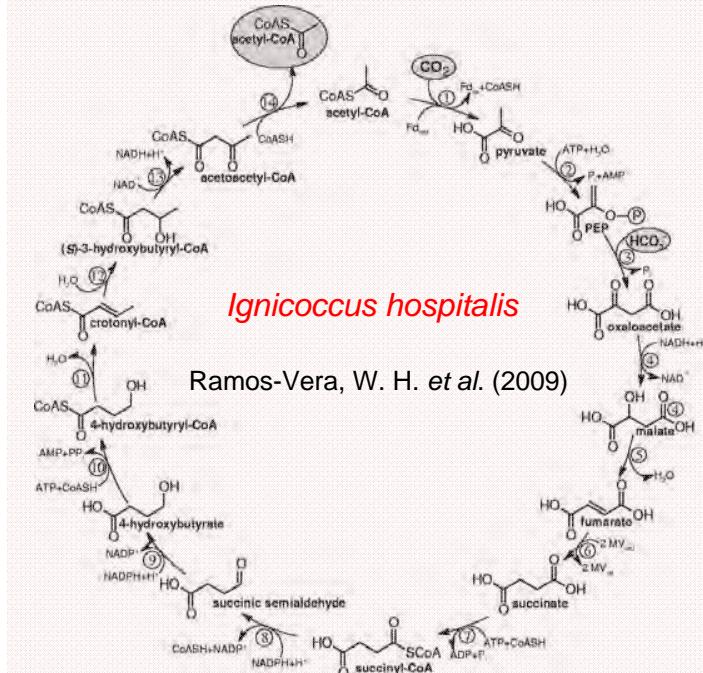
Reductive Acetyl-CoA Pathway



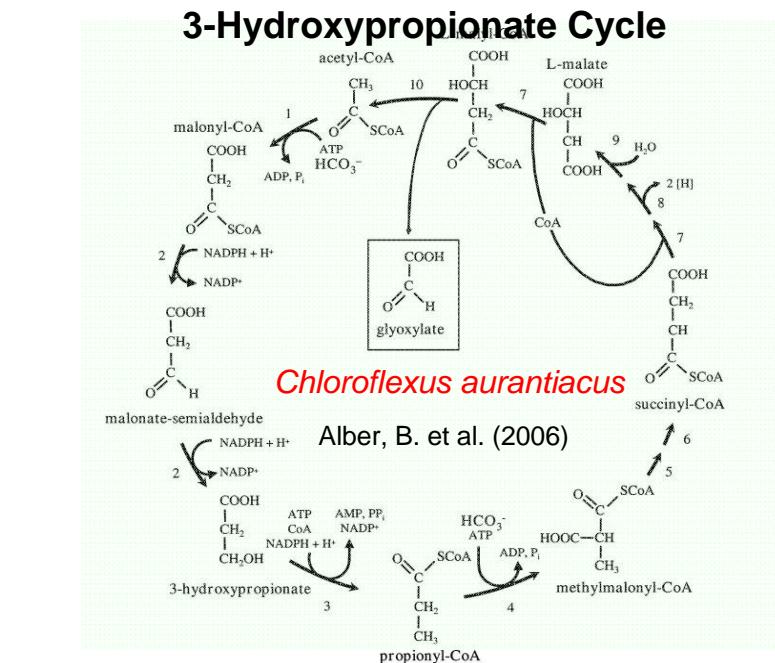
Adapted from <http://lecturer.ukdw.ac.id/dhira/Metabolism/CarbonAssim.html>

Microbial CO₂ Fixation Pathways (Thermophiles)

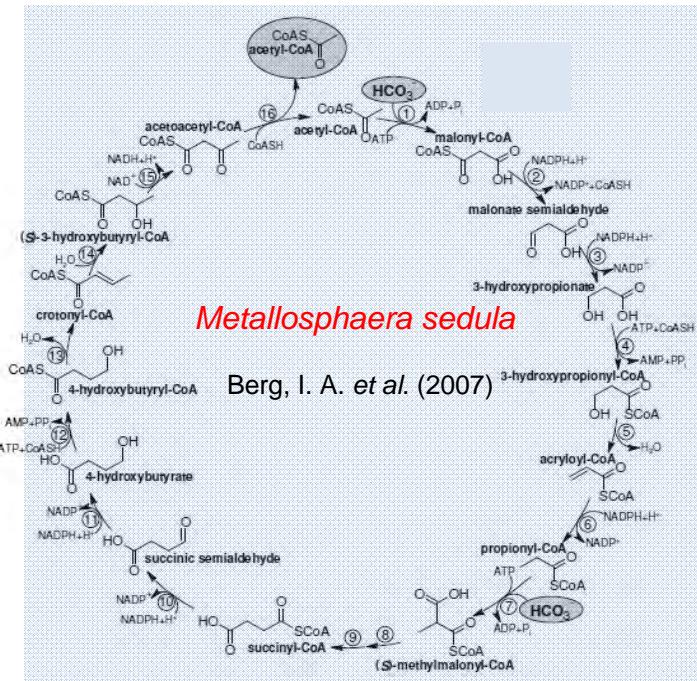
Dicarboxylate/4-Hydroxybutyrate Cycle



Ramos-Vera, W. H. et al. (2009)



3-Hydroxypropionate/4-Hydroxybutyrate Cycle



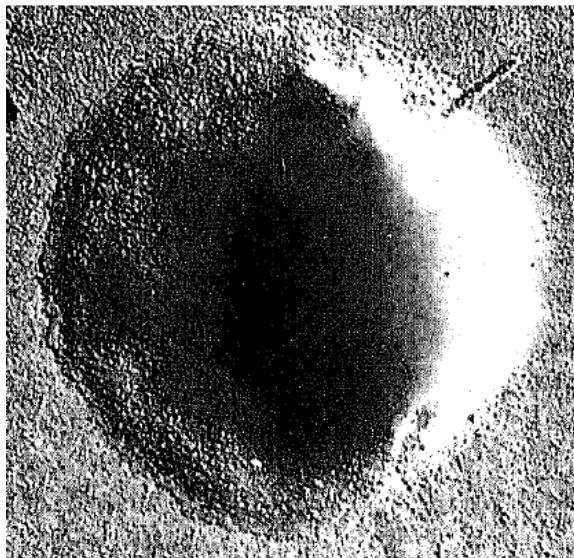
Berg, I. A. et al. (2007)

The Extremely Thermoacidophilic Archaeon *Metallosphaera sedula*

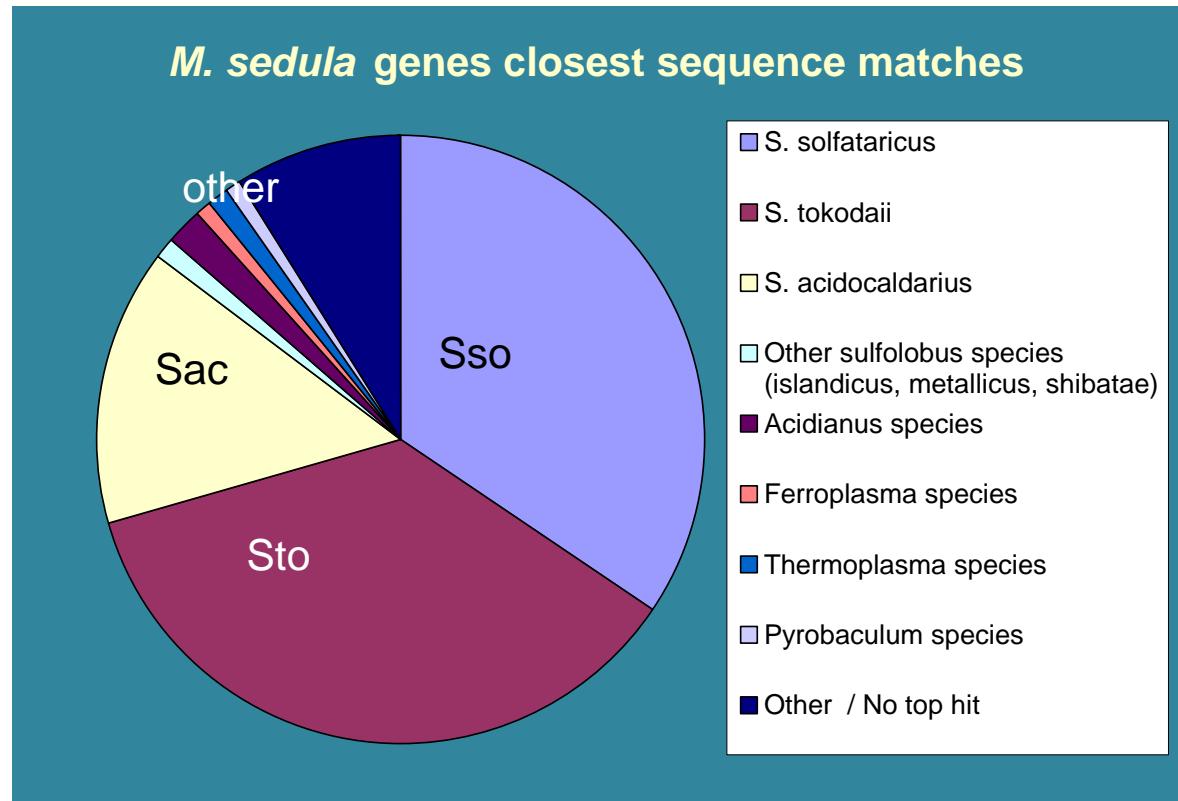
Genome: 2.2 Mb (46.2% G + C) 2304 ORFs

Growth: Mixotrophic aerobe; metal mobilizer

$T_{opt} = 73^{\circ}\text{C}$; $\text{pH}_{opt} = 2.0$



Huber et al. 1989



Auernik, K. S., Y. Maezato, P. H. Blum, and R. M. Kelly. 2008. The genome sequence of the metal-mobilizing, extremely thermoacidophilic archaeon *Metallosphaera sedula* provides insights into bioleaching-associated metabolism. Appl. Environ. Microbiol. 74:682-92.

Comparative genomics: Extreme thermoacidophiles

FUNCTION	COMPONENTS	Ss	St	Sa	Pt	Ms	Cm	Si _L	Si _M	Si _U	Si _{YG}	Ab	Aa	Sm
Terminal Oxidases	SoxABCDD'L	X	X	X	-	X	-	X	X	X	X	-	?	?
	SoxEFGHIM	X	X	X	X	X	X	X	X	X	X	-	?	?
	DoxBCE	X	X	X	X	X	X	X	X	X	X	-	X	?
Iron Oxidation	FoxABCD	-	X	-	-	X	-	-	-	-	-	-	?	X
	Rusticyanin1	-	-	-	-	X	X	X	X	X	X	-	?	?
	Rusticyanin2	-	-	-	-	X	-	-	-	X	X	-	?	?
Sulfur Oxidation	Sor	-	X	-	X	-	-	-	-	-	-	-	X	X
	TetH	-	X	-	X	X	X	X	X	X	X	-	?	?
	DoxDA/TQO	X	X	-	X	X	-	X	X	X	X	-	X	X
CO ₂ Fixation	3-hydr/4-butyry	X	X	X	-	X	-	X	X	X	X	-	?	?
H ₂ Utilization	Hydrogenase (NiFe)	-	-	-	-	X	-	?	-	X	-	X	X	?
Ss (<i>Sulfolobus solfataricus</i>)		St (<i>Sulfolobus tokodaii</i>)				Sa (<i>Sulfolobus acidocaldarius</i>)				Si (<i>Sulfolobus islandicus</i>, strains L,M,U,YG & YN)				
Ms (<i>Metallosphaera sedula</i>)		Cm (<i>Caldivirga maquilingensis</i>)				Sm (<i>Sulfolobus metallicus</i>)				Pt (<i>Picrophilus torridus</i>)				
Ab (<i>Aciduliprofundum boonei</i>)		Aa (<i>Acidianus ambivalens</i>)												

Comparative genomics: Extreme thermoacidophiles

FUNCTION	COMPONENTS	Ss	St	Sa	Pt	Ms	Cm	Si _L	Si _M	Si _U	Si _{YG} N	Ab	Aa	Sm
Terminal Oxidases	SoxABCDD'L	X	X	X	-	X	-	X	X	X	X	-	?	?
	SoxEFGHIM	X	X	X	X	X	X	X	X	X	X	-	?	?
	DoxBCE	X	X	X	X	X	X	X	X	X	X	-	X	?
Iron Oxidation	FoxABCD	-	X	-	-	X	-	-	-	-	-	-	?	X
	Rusticyanin1	-	-	-	-	X	X	X	X	X	X	-	?	?
	Rusticyanin2	-	-	-	-	X	-	-	-	X	X	-	?	?
Sulfur Oxidation	Sor	-	X	-	X	-	-	-	-	-	-	-	X	X
	TetH	-	X	-	X	X	X	X	X	X	X	-	?	?
	DoxDA/TQO	X	X	-	X	X	-	X	X	X	X	-	X	X
CO ₂ Fixation	3-hydr/4-butyry	X	X	X	-	X	-	X	X	X	X	-	?	?
H ₂ Utilization	Hydrogenase (NiFe)	-	-	-	-	X	-	?	-	X	-	X	X	?
Ss (<i>Sulfolobus solfataricus</i>)		St (<i>Sulfolobus tokodaii</i>)		Sa (<i>Sulfolobus acidocaldarius</i>)		Si (<i>Sulfolobus islandicus</i>, strains L,M,U,YG & YN)		Sm (<i>Sulfolobus metallicus</i>)		Pt (<i>Picrophilus torridus</i>)				
Ms (<i>Metallosphaera sedula</i>)		Cm (<i>Caldivirga maquilingensis</i>)												
Ab (<i>Aciduliprofundum boonei</i>)		Aa (<i>Acidianus ambivalens</i>)												

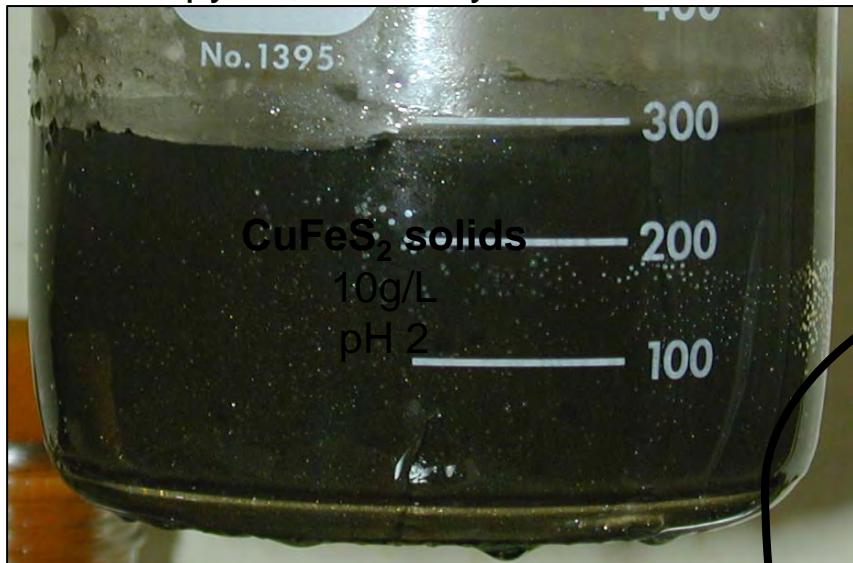
Growing E. coli



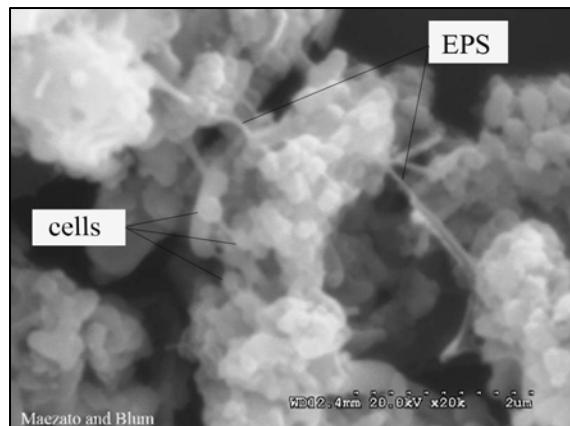
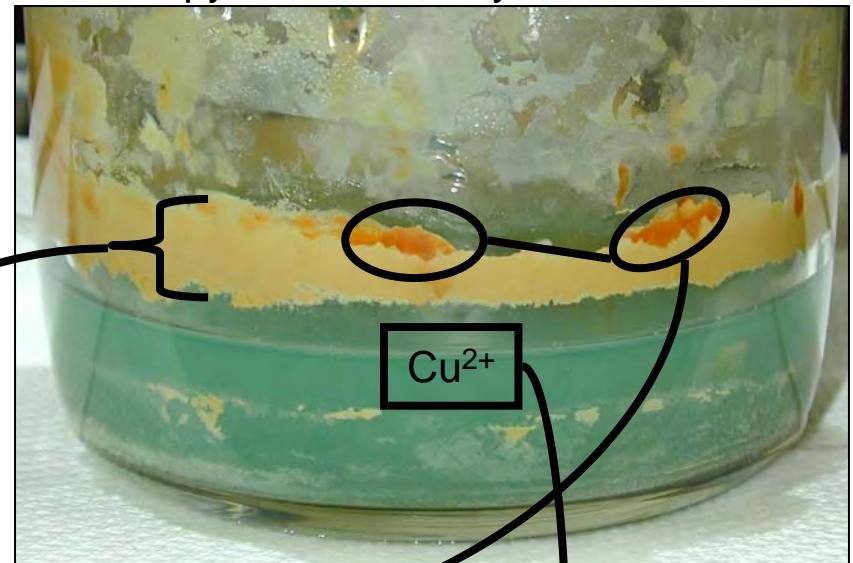
Oxoid

Metal mobilization by *M. sedula*

Chalcopyrite, t = 0 days with *M. sedula*



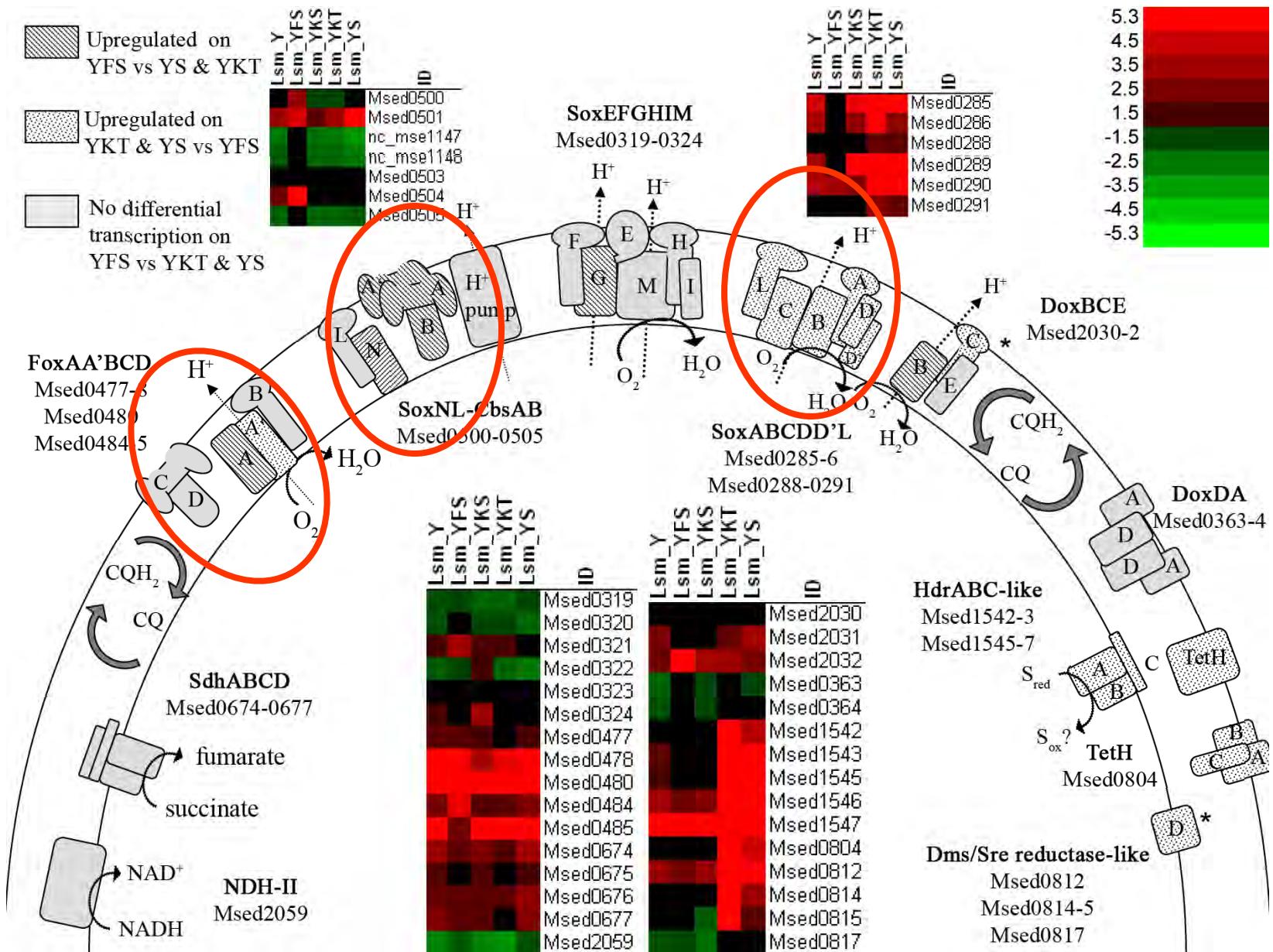
Chalcopyrite, t = 21 days with *M. sedula*



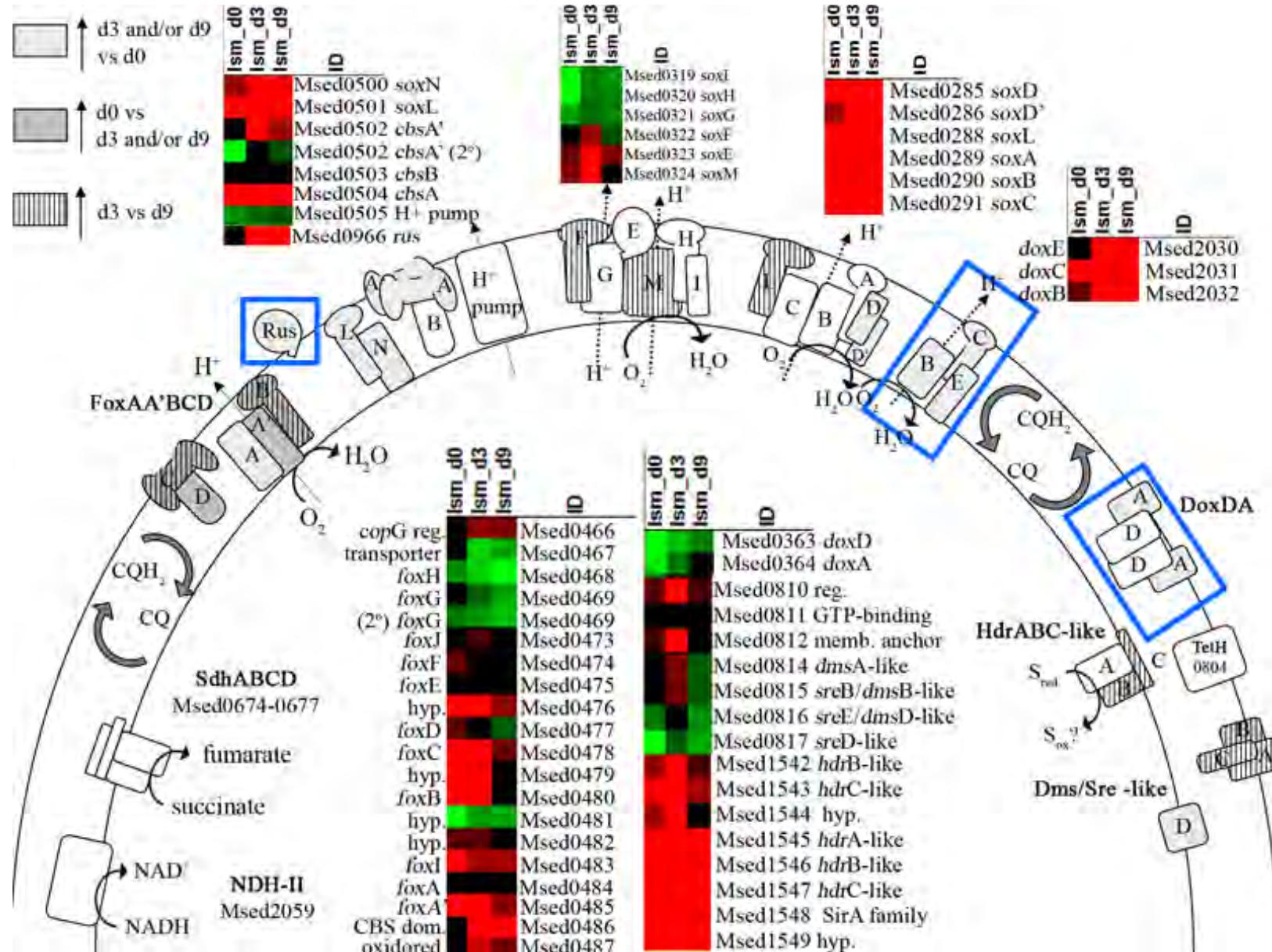
Iron oxidation
Sulfur oxidation
(Inorganic) Carbon fixation
Heavy metal tolerance
Adhesion to solids

Auernik, KS, Y. Maezato, PH Blum, and RM Kelly. 2008. *Appl. Environ. Microbiol.* **74**(3): 682-692
Auernik, KS, CR Cooper, and RM Kelly. 2008. *Curr. Opin. Biotechnol.* **19**(5):445-453

Metal and RISC oxidation components on Fe²⁺, S°

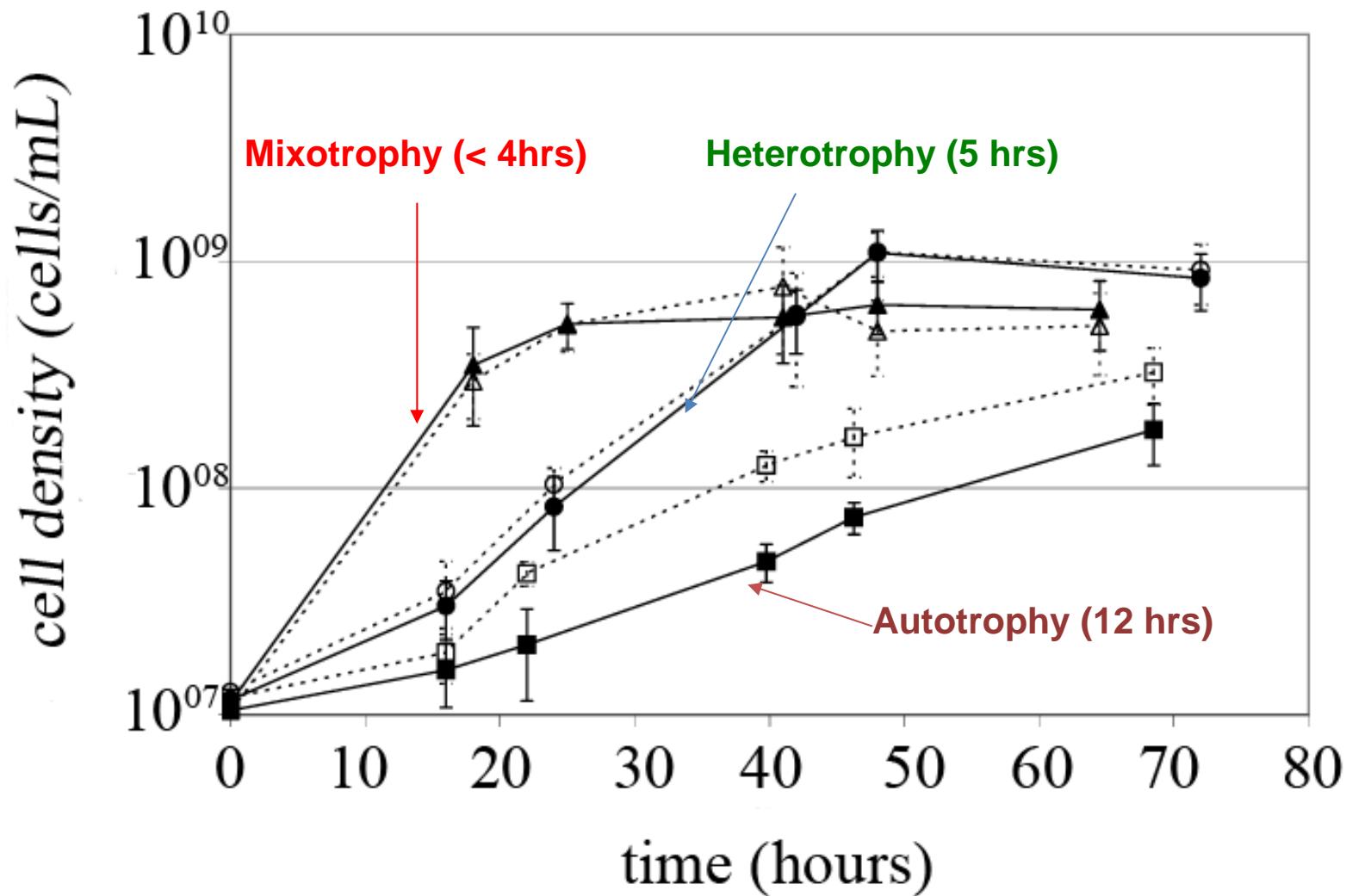


Metal and RISC oxidation components on CuFeS₂



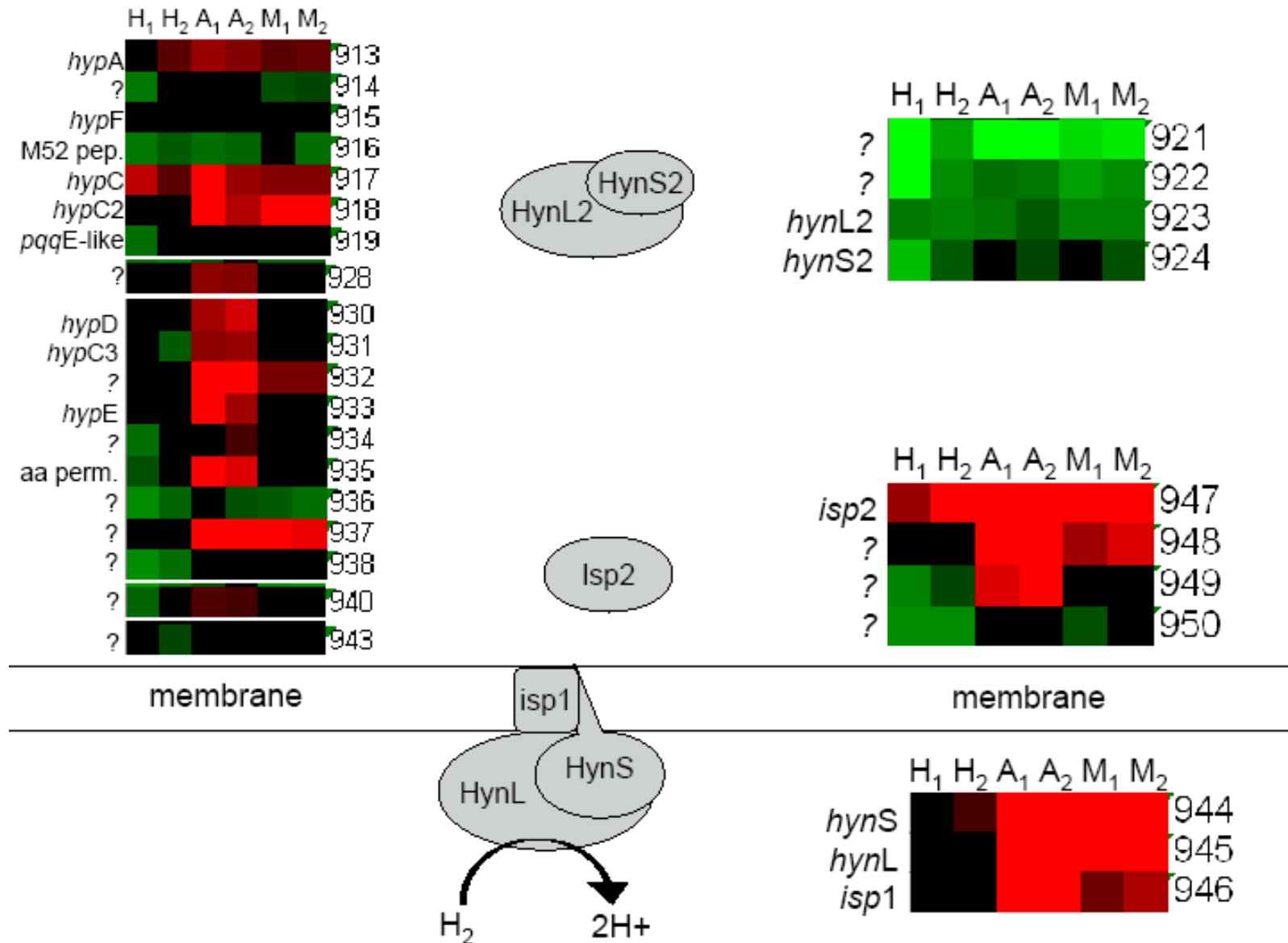
Auernik, KS, and RM Kelly. Molecular hydrogen impacts chalcopyrite bioleaching efficiency for the extremely thermoacidophilic archaeon *Metallosphaera sedula*. (submitted)

Versatile growth physiology of *M. sedula*



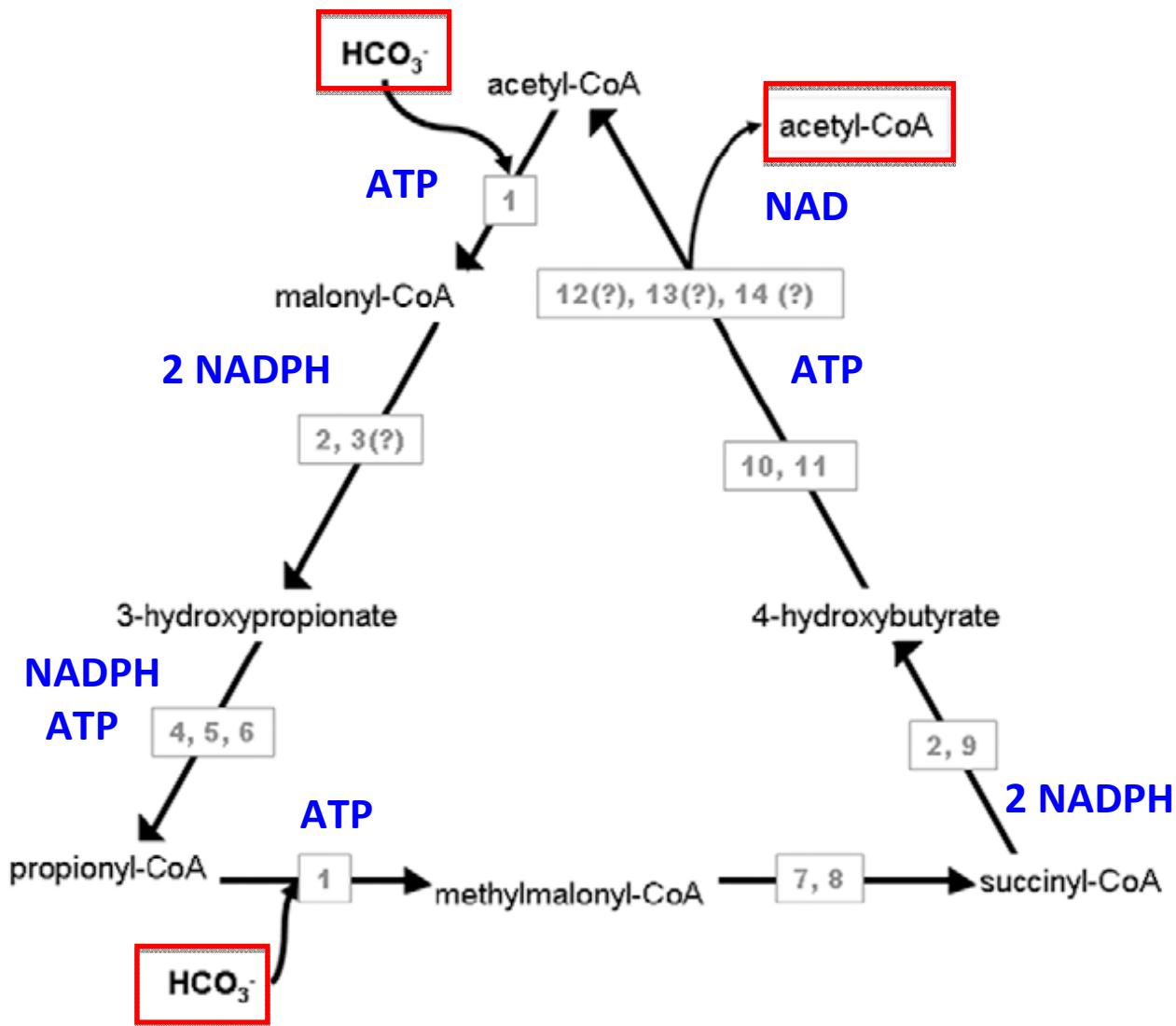
Auernik, KS, and RM Kelly. Physiological versatility of the extremely thermoacidophilic archaeon *Metallosphaera sedula* supported by heterotrophy, autotrophy and mixotrophy transcriptomes (submitted)

M. sedula Hydrogenases



Auernik, KS, and RM Kelly. Physiological versatility of the extremely thermoacidophilic archaeon *Metallosphaera sedula* supported by heterotrophy, autotrophy and mixotrophy transcriptomes (submitted)

M. sedula 3-HP/4-HB CO₂ fixation pathway

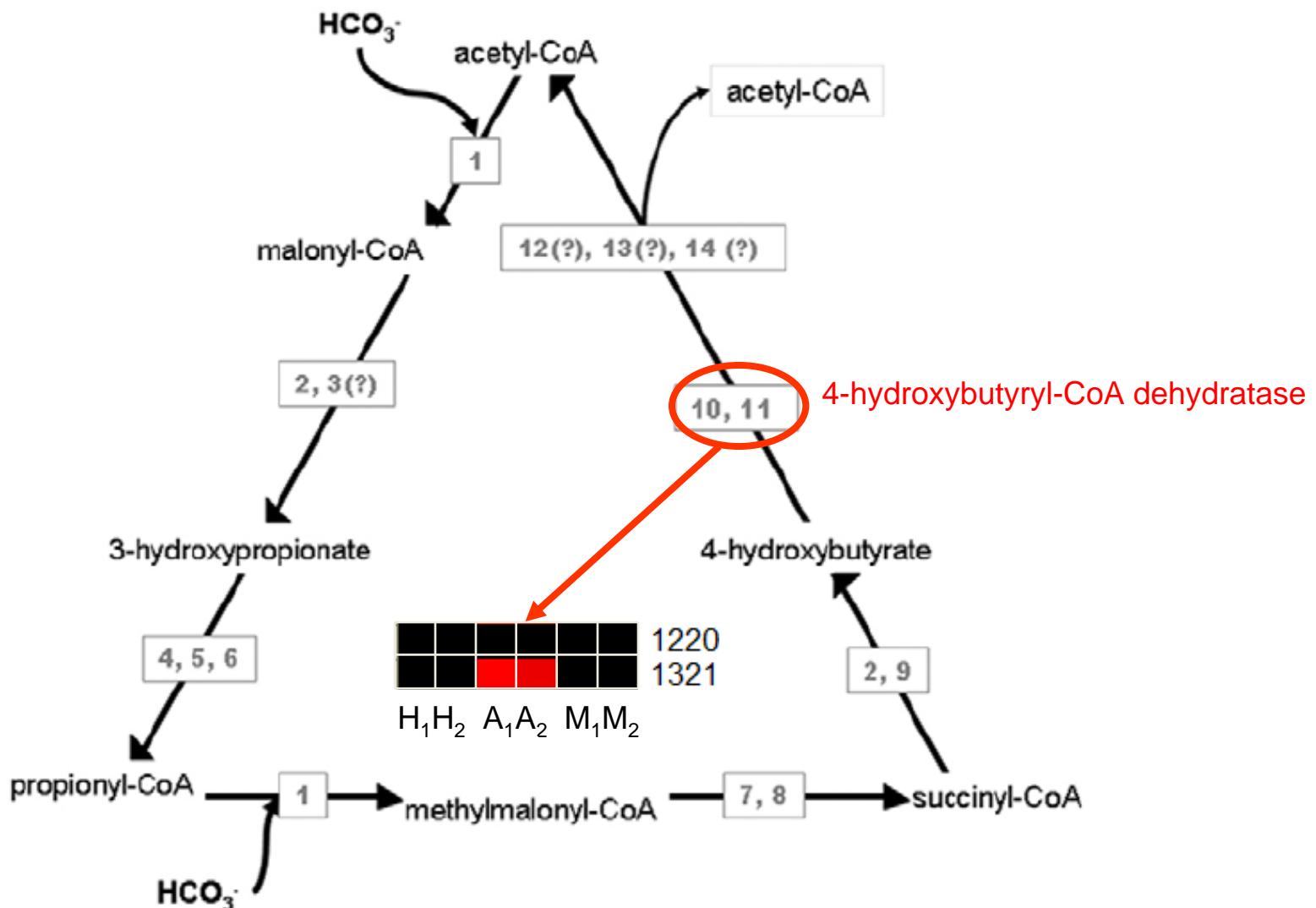


Overall:

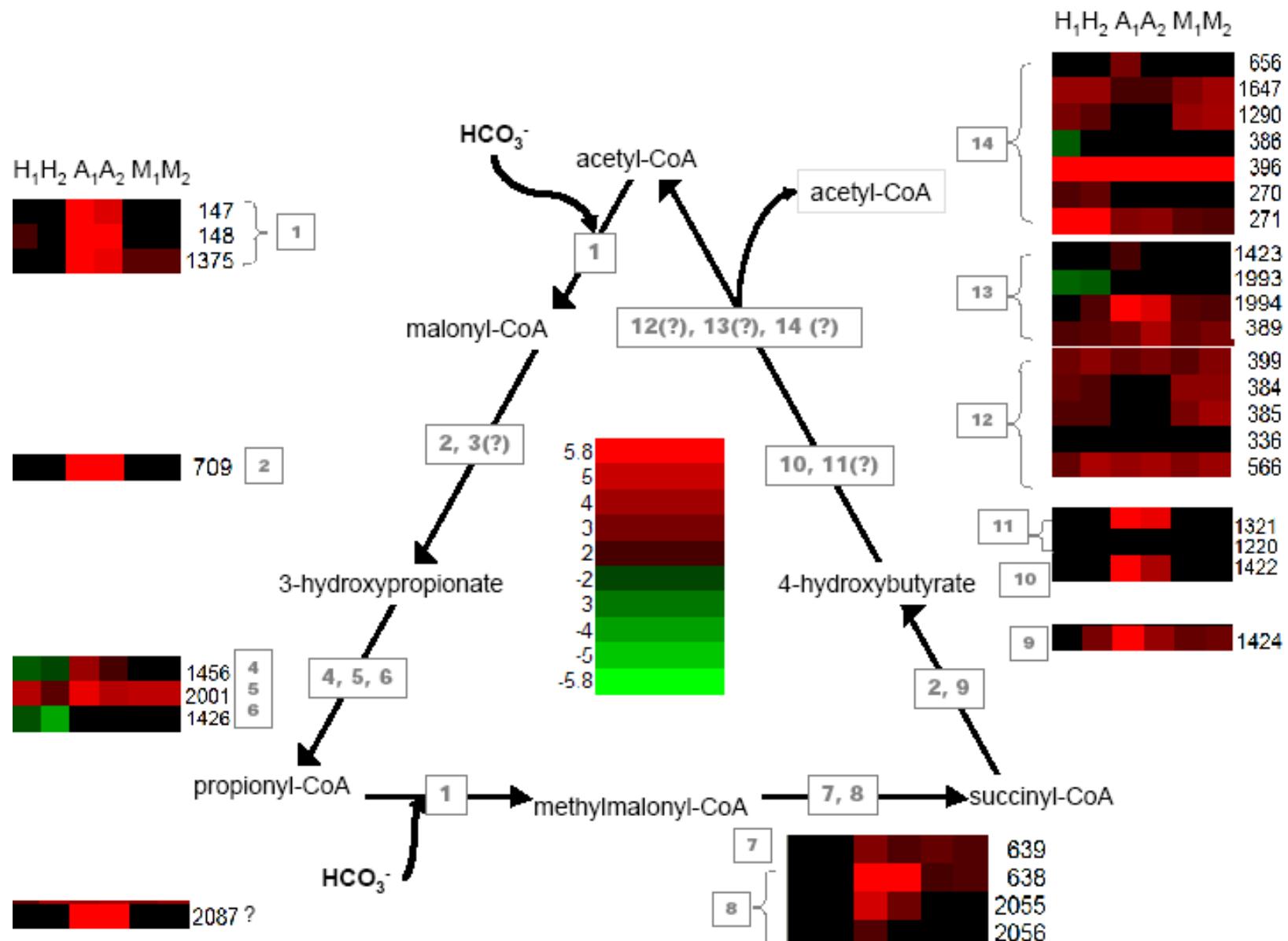


Auernik et al. (2008) *Curr. Opin. Biotech.* 19, 445-453

M. sedula 3-HP/4-HB CO₂ fixation pathway



M. sedula 3-HP/4-HB CO₂ fixation pathway



Auernik, KS, and RM Kelly. Physiological versatility of the extremely thermoacidophilic archaeon *Metallosphaera sedula* supported by heterotrophy, autotrophy and mixotrophy transcriptomes (submitted)

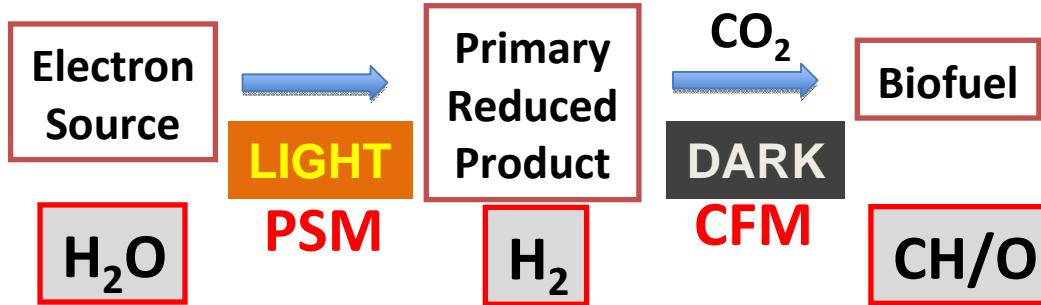
3-HP/4-HB CO₂ Fixation Pathway in *Metallosphaera sedula*

#	Enzyme	Msed Genes	Biochemical confirmation	Comment
1**	Acetyl-CoA/propionyl-CoA carboxylase	Msed_0147,0148,1375	N,R	
2**	Malonyl-CoA/succinyl-CoA reductase	Msed_0709	N,R (Sto)*	NADPH-requiring
3	Malonate semialdehyde reductase	Msed_1993	N (partially purified), R	NADPH-requiring
4	3-Hydroxypropionyl-CoA synthetase	Msed_1456	N,R (Sto)*	AMP-forming
5	3-Hydroxypropionyl-CoA dehydratase	Msed_2001	N,R	
6	Acryloyl-CoA reductase	Msed_1426	N,R (Sto)*	NADPH-requiring
1**	Acetyl-CoA/propionyl-CoA carboxylase	Msed_0147,0148,1375	N,R	
7	Methylmalonyl-CoA epimerase	Msed_0639	N/A	Based on homolog
8	Methylmalonyl-CoA mutase	Msed_0638, 2055	N/A	Based on homolog
2**	Succinyl-CoA reductase	Msed_0709	N,R (Sto)*	NADPH-requiring
9	Succinate semialdehyde reductase	Msed_1424	N (partially purified), R	NADPH-requiring
10	4-Hydroxybutyryl-CoA synthetase	Msed_1422	N (partially purified)	ADP-forming
11	4-Hydroxybutyryl-CoA dehydratase	Candidates: Msed_1220, 1321	N/A	
12	Crotonyl-CoA hydratase	Candidates:Msed 0399, 0384, 0385, 0336, 0566	N/A	(S)-3-hydroxybutyryl-CoA-forming
13	(S)-3-Hydroxybutyryl-CoA dehydrogenase	Candidates: Msed_1423, 0399,1993, 0389	N/A	
14	Acetoacetyl-CoA β -ketothiolase	Candidates: Msed_0656, 1647, 1290, 0396, 0386, 0271, 0270	N/A	NAD-requiring

R = recombinant version characterized; N = native version characterized; * = obtained from *Sulfolobus tokodai*

**note that enzymes 1 and 2 are bi-functional and each catalyze two steps in the cycle

SOLAR BIOFUEL PRODUCTION



Fundamental Studies Needed:

1. Photosystems I and II
2. Hydrogenases
3. CO_2 fixation pathways

Key issues:

1. Mechanisms of catalysis and design of biomimetic systems?
2. Electron transfer pathways *between* the three systems?